TENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 July 2001 (02.07.01)	
International application No. PCT/US00/20214	**Applicant's or agent's file reference** 1445.002
International filing date (day/month/year) 25 July 2000 (25.07.00)	**Priority date** (day/month/year) 28 July 1999 (28.07.99)
Applicant CHIEN, David, Y. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

22 February 2001 (22.02.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Athina Nickitas-Etienne
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

10/046,191 Attach. IDS

NOV 2002

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
CHIRON CORPORATION
Attn. HARBIN, Alisa A.
Intellectual Property - R440
P.O. Box 8097
Emeryville, CA 94662-8097
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)* 29/10/2002	
Applicant's or agent's file reference 1445.002	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 00/ 20214	International filing date *(day/month/year)* 27/07/2000
Applicant CHIRON CORPORATION	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

DOCKETED on/by 11/6/02 Ww
Atty AAH PA
File # 1445.002
Due Date Ext
Final Date 12/29/02 - DLS Ext
(Entered NPR 12/5/02)

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Joannes Vergoosen

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

3NSDOCID: <XSISA220NOENP4_1_>

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped), whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

3NSDOCID: <XSISA220NOENP4_I_>

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
1445.002		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/ 20214	27/07/2000	28/07/1999
Applicant		
CHIRON CORPORATION		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1A

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/20214

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/576 G01N33/543 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 40176 A (ALLANDER TOBIAS ERIK ;PERSSON MATS AXEL ATTERDAG (SE)) 30 October 1997 (1997-10-30) page 5, line 21-30; claims 1,10,33,36 page 53, line 15 -page 54, line 30 page 56, line 4-26 ---	1-15
Y	WO 93 04205 A (ABBOTT LAB) 4 March 1993 (1993-03-04) page 3, line 11 -page 4, line 2 page 7, line 20-30; claims 11,13,14,17,18,21; example 7 ---	1-15
Y	WO 92 13892 A (ABBOTT LAB) 20 August 1992 (1992-08-20) page 7, line 25-30; claims 11,15,16,20,21,24,34 page 12, line 28-33 ---	1-15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 2002	29/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040; Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vadot-Van Geldre, E

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 06488 A (GENELABS TECH INC) 1 April 1993 (1993-04-01) page 10, line 31 -page 11, line 30 page 12, line 13-25 page 41, line 20-35 page 43, line 7-9 page 45, line 33 -page 46, line 2 page 55, line 13-30; examples 19,20; table 3	1-15
Y	FR 2 502 154 A (TREPO CHRISTIAN) 24 September 1982 (1982-09-24) page 5 -page 9 page 11 -page 13 page 15 page 29 -page 30	1-15
Y	US 4 464 474 A (COURSAGET PIERRE L J ET AL) 7 August 1984 (1984-08-07) claims 7,8; example 8	1-15
Y	WO 94 01778 A (CHIRON CORP) 20 January 1994 (1994-01-20) page 2, line 25-28 page 6, line 2-6 page 13, line 12-19; claims; example 4	1-15
Y	WO 99 15898 A (CHIRON CORP) 1 April 1999 (1999-04-01) page 2, line 15-20 page 4, line 23-25 page 5, line 10-23; figure 1; example 1	1-15

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/20214

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9740176	A	30-10-1997	US	2002016445 A1	07-02-2002
			CA	2262423 A1	30-10-1997
			WO	9740176 A1	30-10-1997
			EP	0937153 A1	25-08-1999
			JP	2000509966 T	08-08-2000
WO 9304205	A	04-03-1993	US	5308750 A	03-05-1994
			AT	185606 T	15-10-1999
			AU	2585092 A	16-03-1993
			CA	2115923 A1	04-03-1993
			DE	69230146 D1	18-11-1999
			DE	69230146 T2	25-05-2000
			EP	0603307 A1	29-06-1994
			ES	2139607 T3	16-02-2000
			JP	6510192 T	17-11-1994
			WO	9304205 A1	04-03-1993
WO 9213892	A	20-08-1992	AT	167522 T	15-07-1998
			AU	662517 B2	07-09-1995
			AU	1362792 A	07-09-1992
			CA	2101534 A1	01-08-1992
			DE	69225960 D1	23-07-1998
			DE	69225960 T2	21-01-1999
			DK	569537 T3	06-04-1999
			EP	0569537 A1	18-11-1993
			ES	2118129 T3	16-09-1998
			HK	1012838 A1	06-08-1999
			JP	6505389 T	23-06-1994
			WO	9213892 A1	20-08-1992
WO 9306488	A	01-04-1993	AU	2683792 A	27-04-1993
			WO	9306488 A1	01-04-1993
FR 2502154	A	24-09-1982	FR	2502154 A1	24-09-1982
			AT	15605 T	15-10-1985
			DE	3266294 D1	24-10-1985
			EP	0074986 A1	30-03-1983
			WO	8203330 A1	14-10-1982
US 4464474	A	07-08-1984	DE	3175439 D1	13-11-1986
			EP	0058676 A1	01-09-1982
			IT	1138449 B	17-09-1986
			WO	8200205 A1	21-01-1982
WO 9401778	A	20-01-1994	AT	216779 T	15-05-2002
			AU	685059 B2	15-01-1998
			AU	4662993 A	31-01-1994
			CA	2139645 A1	20-01-1994
			CZ	9500006 A3	13-03-1996
			DE	69331845 D1	06-06-2002
			DE	69331845 T2	14-08-2002
			DK	649537 T3	17-06-2002
			EP	0649537 A1	26-04-1995
			FI	950002 A	27-02-1995
			HU	70473 A2	30-10-1995
			JP	7509060 T	05-10-1995
			NO	950006 A	24-02-1995
			PL	307178 A1	02-05-1995

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/20214

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9401778 A		RU 2126158 C1	10-02-1999
		SK 495 A3	11-07-1995
		WO 9401778 A1	20-01-1994
WO 9915898 A	01-04-1999	AU 9497998 A	12-04-1999
		AU 9498098 A	12-04-1999
		CA 2303123 A1	01-04-1999
		CA 2303165 A1	01-04-1999
		EP 1021719 A1	26-07-2000
		EP 1021723 A1	26-07-2000
		JP 2001517797 T	09-10-2001
		JP 2001517799 T	09-10-2001
		WO 9915898 A1	01-04-1999
		WO 9915901 A1	01-04-1999
		US 6391540 B1	21-05-2002
		US 6261764 B1	17-07-2001
		US 2001039009 A1	08-11-2001

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 16/10, C12N 5/20, G01N 33/576 // C07K 14/18

A3

(11) International Publication Number: **WO 99/50301**

(43) International Publication Date: 7 October 1999 (07.10.99)

(21) International Application Number: PCT/EP99/02154

(22) International Filing Date: 29 March 1999 (29.03.99)

(30) Priority Data:
98870060.5 27 March 1998 (27.03.98) EP

(71) Applicant *(for all designated States except US)*: INNOGENETICS N.V. [BE/BE]; Industriepark Zwijnaarde 7, P.O. Box 4, B–9052 Ghent (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MAERTENS, Geert [BE/BE]; Zilversparrenstraat 64, B–8310 St. Kruis Brugge (BE). DEPLA, Erik [BE/BE]; Burgstraat 58, B–9070 Destelbergen (BE). BUYSE, Marie–Ange [BE/BE]; Burg. Edmond Ronsestraat 23, B–9820 Melsen (BE).

(74) Agent: JACOBS, Philippe; Innogenetics N.V., Industriepark Zwijnaarde 7, P.O. Box 4, B–9052 Ghent (BE).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report:
25 November 1999 (25.11.99)

(54) Title: EPITOPES IN VIRAL ENVELOPE PROTEINS AND SPECIFIC ANTIBODIES DIRECTED AGAINST THESE EPITOPES: USE FOR DETECTION OF HCV VIRAL ANTIGEN IN HOST TISSUE

(57) Abstract

Antibodies to two new epitopes on the HCV envelope proteins were identified which allow routine detection of native HCV envelope antigens, in tissue or cells derived from the host. The new epitopes are: the E1 region aa 307–326 and the N-terminal hyper variable region of E2 aa 395–415. Surprisingly, we characterised an antibody that reacts with various sequences of the hypervariable domain of E2. Specific monoclonal antibodies directed against these epitopes and allowing routine detection of viral antigen are described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
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BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

CLAIMS

1. Use of antibodies specifically binding to the C-terminal region of the HCV E1 protein (aa 227-383) or the N-terminal region of the HCV E2 protein (aa 384-450) for the preparation of a natural HCV protein antigen detection kit.

2. The use of antibody according to claim 1 wherein said antibody specifically binds to one of the following epitopes: -aa 307-326 of HCV E1 protein (SEQ ID 30)
-aa 395-415 of HCV E2 protein (SEQ ID 31).

3. The use of antibodies according to any of claims 1 or 2 wherein said antibody is a monoclonal antibody.

4. The use of antibodies according to any of claims 1 to 3 wherein said antibody is secreted by ECACC deposit having accession number 98031214 or 98031215.

5. The use of a functionally equivalent variant or fragment of an antibody according to any of claims 1 to 4.

6. The monoclonal antibody secreted by the ECACC deposit having accession number 98031214.

7. The functionally equivalent variant or fragment of the antibody according to claim 6.

8. The hybridoma cell line of ECACC deposit having accession number 98031214

9. Method for the detection of natural HCV protein antigens comprising:
-contacting a test sample which may contain HCV protein antigens with an antibody according to any of claims 1 to 4 or with a functionally equivalent variant or fragment of said antibody, to form an antibody-antigen complex, and
-determining said antigen-antibody complex with an appropriate marker.

10. The method according to claim 9, wherein said test sample comprises human cells or tissues.

11. The method according to claim 10, wherein said human cells are peripheral blood cells.

12. The method according to claim 10, wherein said human tissue is liver tissue.

13. An assay kit for the detection of natural HCV protein antigens comprising:

-an antibody according to any of claims 1 to 4, or a functionally equivalent variant or fragment of said antibody, and

-appropriate markers which allow to determine the complexes formed between HCV protein antigens in a test sample with said antibody or a functionally equivalent variant or fragment of said antibody.

INTERNATIONAL SEARCH REPORT

Inv tional Application No
PCT/EP 99/02154

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/10 C12N5/20 G01N33/576 //C07K14/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 13892 A (ABBOTT LAB) 20 August 1992 (1992-08-20) page 2, line 32 -page 3, line 4 page 8, line 21-31 page 12, line 35 -page 13, line 7 page 13; table 2 ---	1-13
X	WO 94 05311 A (DEAKIN RES LTD ;COMIS ALFIO (AU); FISCHER PETER (NO); TYLER MARGAR) 17 March 1994 (1994-03-17) page 9, line 16-32 * seq. ID 8 page 30, line 31 -page 31, line 4 page 16, line 3-7 example 3 --- -/--	1-13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Int .tional Application No
PCT/EP 99/02154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DE 42 09 215 A (BOEHRINGER MANNHEIM GMBH) 7 January 1993 (1993-01-07) page 2, line 40-42 page 2, line 56 page 4, line 55 -page 5, line 1 * page 29 seq.ID 12 claims 1,11	1-13
X	WO 93 18054 A (INNOGENETICS NV) 16 September 1993 (1993-09-16) * page 15 peptide XXb-2 example 23 page 60, line 5-23	1-13
A	WO 96 40764 A (US HEALTH) 19 December 1996 (1996-12-19) page 3, line 13-16 page 15, line 19-35 page 28, line 23 -page 29, line 18	1-13
A	MAERTENS G. ET AL.: "MAPPING OF HUMAN AND MURINE B CELL EPITOPES ON PURIFIED HEPATITS C VIRUS E1 AND E2 PROTEINS" "AASLD ABSTRACTS" HEPATOLOGY, vol. 26, no. 4part2, October 1997 (1997-10), page 186A XP002084941 A232 the whole document	6-8
A	HIRAMATSU N. ET AL.: "IMMUNOHISTOCHEMICAL DETECTION OF HEPATITIS C VIRUS-INFECTED HEPATOCYTES IN CHRONIC LIVER DISEASE WITH MONOCLONAL ANTIBODIES TO CORE, ENVELOPE AND NS3 REGIONS OF THE HEPATITS C VIRUS GENOME" HEPATOLOGY, vol. 16, no. 2, 1992, pages 306-311, XP002084942 abstract page 308, left-hand column, line 27 -right-hand column, line 13	1,9-13
A	CHAN S-W. ET AL.: "HUMAN RECOMBINANT ANTIBODIES SPECIFIC FOR HEPATITIS C VIRUS CORE AND ENVELOPE E2 PEPTIDES FROM AN IMMUNE PHAGE DISPLAY LIBRARY" J.GENERAL VIROLOGY, vol. 77, 1996, pages 2531-2539, XP002084943 page 2532, left-hand column, line 32-35 page 2533; table 1 page 2537, right-hand column, line 18-27	6-8

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INTERNATIONAL SEARCH REPORT

In itional Application No
PCT/EP 99/02154

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	SUZUKI, T. (1) ET AL: "Detection of the E1 protein of HCV in peripheral blood mononuclear cells and in lymphocyte infiltrates in the liver." HEPATOLOGY, (OCT., 1998) VOL. 28, NO. 4 PART 2, PP. 271A. MEETING INFO.: BIENNIAL SCIENTIFIC MEETING OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER AND THE 49TH ANNUAL MEETING AND POSTGRADUATE COURSES OF THE AMERICAN ASSOCIATION FOR TH, XP002116634 the whole document	1-13

3

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int :tional Application No
PCT/EP 99/02154

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9213892	A	20-08-1992	AT	167522	T	15-07-1998
			AU	662517	B	07-09-1995
			AU	1362792	A	07-09-1992
			CA	2101534	A	01-08-1992
			DE	69225960	D	23-07-1998
			DE	69225960	T	21-01-1999
			EP	0569537	A	18-11-1993
			ES	2118129	T	16-09-1998
			JP	6505389	T	23-06-1994
WO 9405311	A	17-03-1994	AU	667578	B	28-03-1996
			AU	4934693	A	29-03-1994
			BR	9306984	A	12-01-1999
			CA	2143823	A	17-03-1994
			CN	1091138	A	24-08-1994
			EP	0667786	A	23-08-1995
			HU	71860	A	28-02-1996
			JP	8500829	T	30-01-1996
			NZ	255256	A	24-02-1997
DE 4209215	A	07-01-1993	AT	171710	T	15-10-1998
			AU	652851	B	08-09-1994
			AU	2197392	A	11-02-1993
			CA	2089576	A	05-01-1993
			DE	59209512	D	05-11-1998
			WO	9301210	A	21-01-1993
			EP	0551460	A	21-07-1993
			EP	0881231	A	02-12-1998
			ES	2123558	T	16-01-1999
			JP	2774872	B	09-07-1998
			KR	9710925	B	02-07-1997
			ZA	9204950	A	03-01-1994
WO 9318054	A	16-09-1993	AT	179716	T	15-05-1999
			AU	671623	B	05-09-1996
			BR	9305435	A	27-12-1994
			CA	2102301	A	07-09-1993
			DE	69324751	D	10-06-1999
			EP	0589004	A	30-03-1994
			EP	0891982	A	20-01-1999
			JP	6505806	T	30-06-1994
			NZ	249838	A	28-10-1996
			NZ	299048	A	24-09-1998
			US	5891640	A	06-04-1999
WO 9640764	A	19-12-1996	AU	6157996	A	30-12-1996
			CA	2221313	A	19-12-1996
			EP	0832114	A	01-04-1998

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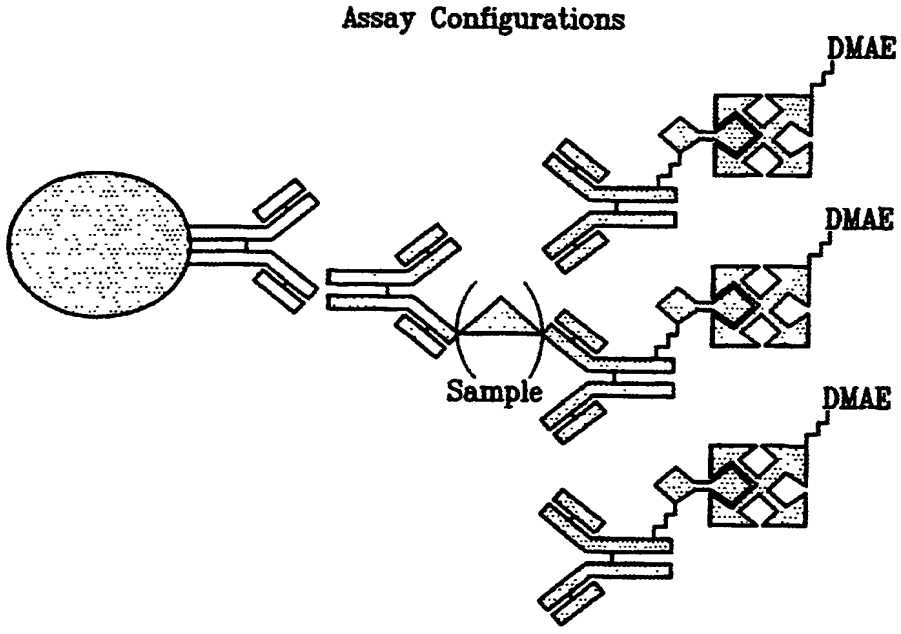
(54) Title: HEPATITIS C VIRAL ANTIGEN IMMUNOASSAY DETECTION SYSTEMS



(57) Abstract: Immunoassays for detecting hepatitis C virus protein and immune complexes between hepatitis C virus protein and antibodies in biological samples, methods of screening blood products for hepatitis C virus, and kits employed therefor are provided.

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HEPATITIS C VIRAL ANTIGEN IMMUNOASSAY DETECTION SYSTEMS

FIELD OF THE INVENTION

The present invention is related generally to immunoassays for detecting hepatitis C virus and specifically to methods of detecting hepatitis C virus in biological samples, methods of screening blood products for hepatitis C virus, and kits therefor.

BACKGROUND OF THE INVENTION

Hepatitis C virus (HCV) was first identified and characterized as the primary cause of post transfusion non-A, non-B hepatitis (NANBH) by Houghton, *et al.*. In addition to providing substantial information concerning HCV, Houghton, *et al.*, and their collaborators have disclosed a number of general and specific immunological reagents and methods. See, e.g. Houghton, *et al.*, EPO Pub. No. 318,216; Houghton, *et al.*, EPO Pub. No. 388,232; Choo, *et al.*, *Science*, **1989**, *244*, 359-362; Kuo, *et al.*, *Science*, **1989**, *244*, 362-364; Takeuchi, *et al.*, *J. Gen. Virol.*, **1990**, *71*, 3027-3033; Takeuchi, *et al.*, *Gene*, **1990**, *91*, 287-291; Takeuchi, *et al.*, *Nucl. Acids Res.*, **1990**, *18*, 4626; Miyamura, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 983-987: Saito, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 6547-6549; Choo, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 2451-2455; Han, *et al.*, *Proc. Natl. Acad.*

Sci. USA, *88*, 1711-1715; Houghton, *et al.*, *Hepatology*, **1991**, *14*, 381-388; and Weiner, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 3468-3472. These publications provide the art with an extensive background on HCV generally, as well as the manufacture and uses of HCV polypeptide immunological reagents. For brevity, therefore, the disclosure of these publications in particular are incorporated herein by reference in their entirety.

Others have readily applied and extended the work of Houghton, *et al.*. See, e.g. Highfield, *et al.*, UK Pat App. 2,239,245 (The Welcome Foundation Ltd.); Wang, EPO Pub. No. 442,394 (United Biomedical Inc.); Leung, *et al.*, EPO Pub. No. 445,423 (Abbott Laboratories); Habits, *et al.*, EPO Pub. No. 451,891 (Akzo N.V.); Reyes, *et al.*, PCT Pub No. WO 91/15516 (Genelabs Inc.); Maki, *et al.*, EPO Pub. No. 468,657 (Tonen Corp.); and Kamada, *et al.*, EPO Pub. No. 469,348 (Shionogi Seiyaku K.K.). See also, Matsuura, *et al.*, *J. Virology*, **1992**, *66*, 1425; Kato, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 9524-9528; Takamizawa, *et al.*, *J. Virol.*, **1991**, *65*, 1105-1113; Chiba, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 4641-4645; Harada, *et al.*, *J. Virol.*, **1990**, *65*, 3015-3021; Hijikata, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 5547-5551; Okamoto, *et al.*, *Jpn. J. Exp. Med.*, **1990**, *60*, 167-177; Yuasa, *et al.*, *J. Gen. Virol.*, **1991**, *72*, 2021-2024; and Watanabe, *et al.*, *Int. J. Cancer*, **1991**, *48*, 340-343.

Sensitive, specific methods for screening and identifying carriers of HCV and HCV-contaminated blood or blood products, as well as monitoring patients undergoing treatment, are important advances in medicine. Post-transfusion hepatitis (PTH) occurs in approximately 10% of transfused patients, and HCV has accounted for up to 90% of these cases. The major problem in this disease is the frequent progression to chronic liver damage (25-55%) relative to other hepatitises, such as type B.

Patient care as well as the prevention of transmission of HCV by blood and blood products or by close personal contact require reliable diagnostic and prognostic tools, such as, for example, HCV antibodies, to detect proteins related to HCV infection. Such antibodies are also useful as agents for monitoring treatment regimens for patients with HCV. Since HCV is a relatively new agent, a continuing need exists to define additional immunological reagents that will allow further study of the clinical course of disease and the epidemiology of HCV in the population.

Current methodology for detecting HCV focuses on detecting HCV-specific antibodies. See, for example, Hada, *et al.*, *Acta Med. Okayama*, **1992**, *46*, 365-70; Miyamura, *et al.*, EPO Pub. No. 0537626; Lok, *et al.*, *Hepatology*, **1993**, *18*, 497-502; Wang, *et al.*, *Vox Sang*, **1992**, *62*, 21-4; Kleinman, *et al.*, *Transfusion*, **1992**, *32*, 805-813; Leon, *et al.*, *Vox Sang*, **1996**, *70*, 213-16; Lesniewski, *et al.*, *J. Med. Virol.*, **1995**, *45*, 415-22; and Inoue, *et al.*, *J. Gen. Virol.*, **1992**, *73*, 2151-54. A major disadvantage to detecting antibodies which react with HCV is that seroconversion has already occurred and the patient may already have a well-established viral infection. Alternatively, if an individual is determined to be HCV antibody reactive, it may simply mean that the individual has been exposed to HCV sometime in the past and may not be presently infected.

Other methods for detecting HCV comprise using PCR. See, for example, Francois, *et al.*, *J. Clin. Microbiol.*, **1993**, *31*, 1189-93. HCV envelope proteins have also been detected by immunohistochemical analysis of hepatocytes in patients with chronic liver disease. However, these assays do not easily lend themselves to a clinical setting. Hiramatsu, *et al.*, *Hepatology*, **1992**, *16*, 306-311.

Other methods also are directed to detecting HCV core protein. See, for example, Orito, *et al.*, *Gut*, **1996**, *39*, 876-80 and Kashiwakuma, *et al.*, *J. Immunol. Methods*, **1996**, *190*, 79-89. These methods include a protein-capture fluorescence enzyme immunoassay (FEIA), a traditional sandwich ELISA assay, using monoclonal antibodies reactive with recombinant HCV core protein. The method consists of using one monoclonal antibody coated on a solid phase as a capture antibody and beta-D-galactosidase conjugated monoclonal antibody as the antigen detection signal antibody. These assays, however, require very tedious sample preparation procedures including, for example, polyethyleneglycol precipitation, NaOH denaturation, retritration of the sample to neutral pH and addition of Triton X-100 to the sample preparation prior to starting the assay. Such assays are not convenient in the clinical setting. Indeed, what is required is an easy, fast immunoassay for detection of HCV antigens.

Applicants have developed an immunoassay system which can detect HCV envelope antigens, E1 and E2, without tedious sample preparation procedures. Applicants' invention provides a means to detect free HCV antigens prior to seroconversion, antigen disappearance after acute infection, and/or interferon therapy and, thus, is useful as a

diagnostic and for blood screening and evaluating the effectiveness of drug treatment. Thus, Applicants' invention is a significant improvement for detecting HCV in biological samples.

SUMMARY OF THE INVENTION

The present invention is directed to methods for detecting hepatitis C virus in biological samples comprising contacting the sample with an anti-human antibody and at least one monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between the antibodies and the sample, and detecting the presence of immune complexes of the antibodies and the envelope protein.

The present invention is also directed to methods for detecting hepatitis C virus in a biological sample comprising contacting an anti-human antibody attached to a solid phase with a polyclonal anti-hepatitis C virus envelope protein antibody, contacting the sample to the polyclonal antibody, contacting the sample with at least one detectably-labeled, monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between the antibodies and the sample, and detecting the presence of immune complexes of the antibodies and the envelope protein and/or the presence of free envelope protein.

The present invention is also directed to methods of screening blood components or blood for hepatitis C virus prior to the use of such blood or blood component to prepare blood products comprising reacting a body component from a potential donor with an anti-human antibody and at least one monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between the antibodies and the body component, detecting the presence of immune complexes formed between the antibodies and hepatitis C virus envelope proteins, and discarding any blood or blood component from the donor if the complexes are detected.

The present invention is also directed to kits for detecting hepatitis C virus in a biological sample comprising an anti-human antibody, at least one monoclonal anti-hepatitis C virus envelope protein antibody, control standards, and instructions for use of the kit components.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A, 1B, 1C represent preferred assay configurations of the present invention. Figure 1D shows preferred components in Figures 1A, 1B, and 1C.

Figure 2 is a graph showing the treatment regimen of a patient having HCV.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The practice of the present invention will employ, unless otherwise indicated, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Ed., 1989; *DNA Cloning: A Practical Approach*, Vols. I & II, D. Glover, Ed.; *Methods In Enzymology*, S. Colowick and N. Kaplan, Eds., Academic Press Inc.; *Handbook of Experimental Immunology*, Vols. I-IV, D.M. Weir and C.C. Blackwell, Eds., Blackwell Scientific Publications; and *Fundamental Virology*, 2nd Ed., Vols. I & II, B.N. Fields and D.M. Knipe, Eds., each of which is incorporated herein by reference in its entirety. In addition, antibodies are prepared following standard published protocols set forth in, for example, Harlow and Lane, 1988, Antibodies: A laboratory manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, which is incorporated herein by reference in its entirety.

"HCV envelope protein" refers to a polypeptide or polypeptide analog (e.g., mimitopes) comprising an amino acid sequence (and/or amino acid analogs) defining at least one HCV epitope within an envelope protein. Typically, the sequences defining the epitope correspond to the amino acid sequence of an HCV protein (either identically or via substitution of analogs of the native amino acid residue that do not destroy the epitope). In general, the epitope-defining sequence will be 5 or more amino acids in length, more typically 8 or more amino acids in length, and even more typically 10 or more amino acids in length.

"Linear epitope" refers to a portion of an envelope protein that comprises a series of contiguous amino acids. The antibody binding portion preferably interacts with an epitope defined by 5 or more contiguous amino acids, more typically 8 or more contiguous amino acids, and even more typically 10 or more contiguous amino acids.

"Linear neutralizing epitope" refers to a linear epitope such that when an antibody is bound to the epitope, the antibodies block viral infection of the target cell.

Antibodies reactive with these epitopes within an envelope protein are able to inhibit or abrogate viral infection of target cells.

"Conformational epitope" refers to epitopes formed by the three-dimensional shape of the antigen (e.g., folding). The length of the epitope defining sequence can be subject to wide variations. Thus, the amino acids defining the epitope can be relatively few in number, but widely dispersed along the length of the molecule (or even on different molecules in the case of dimers, etc.), being brought into the correct epitope conformation via folding. The portions of the antigen between the residues defining the epitope may not be critical to the conformational structure of the epitope. For example, deletion or substitution of these intervening sequences may not affect the conformational epitope provided sequences critical to epitope conformation are maintained (e.g., cysteines involved in disulfide bonding, glycosylation sites, etc.).

"E1" as used herein refers to a protein or polypeptide expressed within the first 400 amino acids of an HCV polyprotein, sometimes referred to as the E or S protein. In its natural form it is a 35 kD glycoprotein which is found in strong association with membrane. In most natural HCV strains, the E1 protein is encoded in the viral polyprotein following the C (core) protein. The E1 protein extends from approximately amino acid (aa) 192 to about aa 383 of the full-length polyprotein. The term "E1" as used herein also includes analogs and truncated forms that are immunologically cross-reactive with natural E1.

"E2" as used herein refers to a protein or polypeptide expressed within the first 900 amino acids of an HCV polyprotein, sometimes referred to as the NS1 protein. In its natural form it is a 72 kD glycoprotein that is found in strong association with membrane. In most natural HCV strains, the E2 protein is encoded in the viral polyprotein following the E1 protein. The E2 protein extends from approximately aa 384 to about aa 820. The term "E2" as used herein also includes analogs and truncated forms that are immunologically cross-reactive with natural E2.

The term "aggregate" as used herein refers to a complex of E1 and/or E2 containing more than one E1 or E2 monomer. E1:E1 dimers, E2:E2 dimers, and E1:E2 heterodimers are all "aggregates" within the scope of this definition. Aggregates may also include larger forms, and may have molecular weights in excess of 800 kD.

The phrase "biological sample" refers to a fluid or tissue of a mammal (e.g., an anthropoid, a human) that commonly contains antibodies or viral particles. Such components are known in the art and include, without limitation, blood, plasma, serum, spinal fluid, lymph fluid, secretions of the respiratory, intestinal or genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas. Biological samples also include biological liquids. The term "biological liquid" refers to a fluid obtained from an organism. Some biological liquids are used as a source of other products, such as clotting factors (e.g., Factor VIII:C), serum albumin, growth hormone, and the like. In such cases, it is important that the source of biological liquid be free of contamination by virus such as HCV. Biological samples are also referred to as "body components."

The phrase "solid phase" refers to a solid body to which the anti-human antibody is bound covalently or by noncovalent means such as hydrophobic adsorption. The solid phases facilitate separation of the sample from the antibody after incubation. Preferred examples of solid phases that can be used are nitrocellulose (e.g., in membrane or microtiter well form), polyvinyl chloride (e.g., in sheets or microtiter wells), polystyrene latex (e.g., in beads or microtiter plates, polyvinylidine fluoride (known as Immunlon™)), diazotized paper, nylon membranes, activated beads, Protein A beads, magnetic latex particles (MLP), paramagnetic particles (PMP), and paramagnetic beads. For example, Dynatech Immunlon™ 1 or Immunlon™ 2 microtiter plates or 0.25 inch polystyrene beads (Precision Plastic Ball) can be used.

One aspect of the present invention is directed to methods for detecting hepatitis C virus in a biological sample. Numerous immunoassay formats can be used according to the present invention. The immunoassay format, however, must allow for interaction among the components, *i.e.*, the antibodies and the proteins that may be present in the biological sample. A preferred immunoassay format is an ELISA antigen-capture assay described below in greater detail. Equivalent immunoassay formats, however, are known to those skilled in the art and are included within the scope of the invention.

In a preferred embodiment of the invention, a method of detecting immune complexes of hepatitis C virus and antibody in a patient comprises contacting a biological sample with an anti-human antibody and at least one monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between the

antibodies and the sample, and detecting the presence of immune complexes of the antibodies and the envelope protein which may be present in the biological sample. A preferred embodiment of such an assay configuration is shown in Figure 1B. This assay configuration can detect immune complexes comprising human anti-HCV antibodies bound to E1 and/or E2 proteins present in biological samples.

In another preferred embodiment of the present invention, a polyclonal anti-hepatitis C envelope protein antibody is contacted to the anti-human antibody, prior to contacting the biological sample, under conditions which allow an immunologic reaction between the anti-human antibody and the polyclonal anti-hepatitis C envelope protein antibodies. Preferred embodiments of such an assay configuration are shown in Figures 1A and 1C. This assay configuration can detect free envelope antigen (Figure 1A) as well as immune complexes comprising human anti-HCV antibodies bound to E1 and/or E2 proteins (Figure 1C).

The anti-human antibody can be commercially obtained from several sources including, for example, Boehringer Mannheim. Any anti-human antibody can be used in the present invention and can be derived from any animal or can be synthetically prepared. A preferred antibody is mouse anti-human IgG Fc obtained from Walpole. Such antibodies are also able to bind primate antibodies, such as, for example, primate polycolonal anti-hepatitis C envelope protein antibodies. Preferably, the anti-human antibody is attached to a solid phase by standard techniques known to those skilled in the art. Preferably, the solid phase is a microtiter plate, paramagnetic particles or paramagnetic beads. Preferably, the anti-human antibodies are diluted to optimal concentration in PBS (pH 7.4) and coated on PMP.

The polyclonal anti-hepatitis C envelope protein antibodies can be generated using standard antibody generation techniques well known to those skilled in the art. These polyclonal antibodies preferably are recognized by the anti-human antibody. Accordingly, the polyclonal antibodies are preferably generated using primates such as, for example, chimpanzees. Preferably, as described below in greater detail, the primate is immunized with an e1/e2 heterodimer.

The anti-human antibody/primate polyclonal anti-HCV antibody complex bound to the solid phase is preferably contacted with the biological sample under conditions which allow an immunologic reaction between the polyclonal anti-HCV antibody and the free

E1 and/or E2 proteins or E1 or E2 immune complexes, if present, in the biological sample. Such conditions will preferably be under physiologic temperature, pH and ionic strength and can take place in media such as, for example, phosphate buffered saline (PBS). Preferably, biological samples, which may be diluted from 0-fold to 1000-fold in sample diluent, are incubated for about 20 minutes to about 1 hour at 37°C with the PMP-bound anti-human antibodies, then washed with wash buffer in connection with retention by a magnetic source.

After the biological sample is contacted with either the anti-human antibody (Figure 1B) or the polyclonal anti-HCV envelope protein antibodies (Figures 1A or 1C), the biological sample is further contacted with at least one monoclonal antibody which reacts with an epitope of either E1 or E2 under conditions which allow an immunologic reaction between the monoclonal anti-E1/E2 antibodies and the E1 and/or E2 proteins, if present, in the biological sample. Such conditions will preferably be under physiologic temperature, pH and ionic strength and may take place in media such as, for example, phosphate buffered saline (PBS). Preferably, the epitopes include an e2 conformational epitope, an e2 linear epitope, an e2 neutralizing epitope, an e1 conformational epitope, an e1 linear epitope, or an e1 neutralizing epitope. The neutralizing epitopes can be either linear or conformational. In preferred embodiments of the invention, the biological sample is contacted with a combination of different monoclonal antibodies reactive with these epitopes. The combination of monoclonal antibodies can include all of the possible combinations of the above-described epitopes. Monoclonal antibodies reactive with the above-described epitopes can be prepared by one skilled in the art using standard antibody production techniques. The mixture of the bound biological sample and monoclonal antibodies is preferably incubated for about 20 minutes to about 1 hour at 37°C, then washed with wash buffer in connection with retention by a magnetic source.

In order to prepare monoclonal antibodies to E1 and E2, the envelope antigens are prepared. The E1 domain, which is believed to correspond to the viral envelope protein, is currently estimated to span amino acids 192-383 of the HCV polyprotein (PCT Pub. No. WO91/15771, which is incorporated herein by reference in its entirety). Upon expression in a CHO system (glycosylated), it is believed to have an approximate molecular weight of 35 Kd as determined via SDS-PAGE. The E2 protein, previously called NS1, is believed to span amino acids 384-800 of the polyprotein and to also be an envelope protein. Upon expression

in a CHO system (glycosylated), it is believed to have an apparent gel molecular weight of about 72 Kd. It is understood that these protein endpoints are approximations (e.g., the carboxy terminal of E2 could lie somewhere in the 750-820 amino acid region). It is also understood that the prototype isolate HCV1 sequence in the aforementioned PCT application is cited for illustrative purposes only and that any HCV isolate (see, e.g., references cited in the "Background" section) is a suitable source of E1 and/or E2 sequence for the practice of the present invention.

The E1 and E2 proteins used in the present invention to elicit antibody production can be full-length viral proteins, substantially full-length versions thereof, or functional fragments thereof (e.g., fragments which are not missing sequence essential to the formation or retention of a conformational epitope). The HCV proteins of the present invention can be made by any convenient method that provides the epitope of interest. For example, recombinant expression in mammalian or insect cells is a preferred method to provide secreted glycosylated E1 and/or E2 antigens in "native" conformation. However, it may also be possible, as it is known for proteins, to express the antigens in other recombinant hosts and renature the protein after recovery. It is also understood that chemical synthesis may also provide conformational antigen mimitopes that cross-react with the "native" antigen's conformational epitope.

Complexes of E1 and/or E2 (also called aggregates) containing more than one E1 or E2 monomer are also preferred antigens. E1:E1 dimers, E2:E2 dimers, and E1:E2 heterodimers are all antigens within the scope of this invention. Aggregates may also include larger forms, and may have molecular weights in excess of 800 kD.

Fusion polypeptides including a polypeptide in which the HCV antigen(s) are part of a single continuous chain of amino acids, which chain does not occur in nature, can also be used to prepare monoclonal antibodies. The HCV antigens may be connected directly to each other by peptide bonds or be separated by intervening amino acid sequences. The fusion polypeptides may also contain amino acid sequences exogenous to HCV.

Methods for preparing E1 and E2 antigens including those with native conformations are described in Spaete, *et al.*, *Virology*, **1992**, *188*, 819-830, and in WO 92/08734 and in U.S. Serial No. 07/758,880, which are incorporated herein by reference in their entirety. Generally, host cells are chosen that will allow the formation of native

conformational epitopes within the expressed envelope proteins; these host cells may include, for example, animal cells, insect cells, yeast cells, and the like.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include viral promoters such as that from Simian Virus 40 (SV40) (Fiers, *Nature*, **1978**, *273*, 113), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly A addition sequences; enhancer sequences which increase expression may also be included, and sequences which cause amplification of the gene may also be desirable. These sequences are known in the art.

Vectors suitable for replication in mammalian cells are known in the art, and may include viral replicons, or sequences which ensure integration of the appropriate sequences encoding NANBV epitopes into the host genome.

A vector which is used to express foreign DNA, and which may be used in vaccine preparation is Vaccinia virus. In this case the heterologous DNA is inserted into the Vaccinia genome. Techniques for the insertion of foreign DNA into the vaccinia virus genome are known in the art, and utilize, for example, homologous recombination. The insertion of the heterologous DNA is generally into a gene which is non-essential in nature, for example, the thymidine kinase gene (tk), which also provides a selectable marker. Plasmid vectors that greatly facilitate the construction of recombinant viruses have been described (see, for example, Mackett, *et al.*, *J. Virol.*, **1984**, *49*, 857, Chakrabarti, *et al.*, *Mol. Cell Biol.*, **1985**, *5*, 3403; and Moss in GENE TRANSFER VECTORS FOR MAMMALIAN CELLS, Miller and Calos, eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., **1987**, p. 10.). Expression of the HCV polypeptide then occurs in cells or individuals which are immunized with the live recombinant vaccinia virus.

The segment of HCV CDNA encoding the desired sequence is inserted into a Vaccinia vector. The polypeptide encoding sequence may be attached to a leader sequence. The leader sequence may be that for tissue plasminogen activator (TPA), or from another source, e.g., that for beta-globin. The heterologous polynucleotide may be inserted into a

vaccinia vector which is a modified version of pSC11, due to the addition of a polylinker sequence which contains a cloning site.

In order to detect whether or not the HCV polypeptide is expressed from the vaccinia vector, BSC 1 cells may be infected with the recombinant vector and grown on microscope slides under conditions which allow expression. The cells may then be acetone-fixed, and immunofluorescence assays performed using serum which is known to contain anti-HCV antibodies to a polypeptide(s) encoded in the region of the HCV genome from which the HCV segment in the recombinant expression vector was derived.

Other systems for expression of E1 and E2 include insect cells and vectors suitable for use in these cells. These systems are known in the art, and include, for example, insect expression transfer vectors derived from the baculovirus Autographa californica nuclear polyhedrosis virus (AcNPV), which is a helper-independent, viral expression vector. Expression vectors derived from this system usually use the strong viral polyhedrin gene promoter to drive expression of heterologous genes. Currently the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed for improved expression. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; See Luckow and Summers, *Virology*, **1989**, *17*, 31). Good expression of nonfused foreign proteins usually requires foreign genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. The plasmid also contains the polyhedrin polyadenylation signal and the ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Methods for the introduction of heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summer and Smith, Texas Agricultural Experiment Station Bulletin No. 1555; Ju, *et al.* (1987) ; Smith, *et al.*, *Mol. & Cell Biol.*, **1983**, *3*, 2156-2165; and Luckow and Summers, *Virology*, **1989**, *17*, 31). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. The inserted sequences may be those which encode all or varying segments of the polyprotein, or other ORFs which encode viral polypeptides. The signals for posttranslational

modifications, such as signal peptide cleavage, proteolytic cleavage, and phosphorylation, appear to be recognized by insect cells. The signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells. Examples of the signal sequences from vertebrate cells which are effective in invertebrate cells are known in the art, for example, the human interleukin 2 signal ($IL2_S$) which is a signal for transport out of the cell, is recognized and properly removed in insect cells.

Once the E1 and E2 proteins have been prepared, such as by the methods described above, monoclonal antibodies to conformational epitopes and linear epitopes can be prepared using standard monoclonal antibody techniques. Monoclonal antibodies directed to conformational epitopes of E1 and E2 are prepared using intact, native E1 and E2 proteins. Monoclonal antibodies directed to linear epitopes of E1 and E2 are prepared using denatured E1 and E2 proteins. The presence or absence of a conformational epitope can be readily determined through screening the E1 or E2 protein with an antibody and comparing its reactivity to that of a denatured version of the antigen which retains only linear epitopes (if any). In such screening using polyclonal antibodies, it may be advantageous to adsorb the polyclonal serum first with the denatured E1 or E2 protein and see if it retains antibodies to the E1 or E2 protein. Preferably, the monoclonal antibody preparations are compositions wherein the desired antibody comprises at least 35% of the total protein component in the composition. The desired antibody preferably comprises at least 40%, more preferably at least about 50%, more preferably at least about 60%, still more preferably at least about 70%, even more preferably at least about 80%, even more preferably at least about 90%, and most preferably at least about 95% of the total protein component. The composition can contain other compounds such as carbohydrates, salts, lipids, solvents, and the like, without affecting the determination of the percentage purity as used herein.

Preferred monoclonal antibodies to conformational epitopes include, but are not limited to, 5E5/H7 (IgG1 anti-HCV e2 prepared from amino acids 1-967 of e1/e2 in HeLa cells), 2A3/B12 (anti-HCV e2 prepared from amino acids 1-967 of e1/e2 in HeLa cells), 5E9/D10 (anti-HCV e2 prepared from amino acids 1-967 of e1/e2 in HeLa cells), 3F5/H6 (anti-HCV e2 prepared from amino acids 1-967 of e1/e2 in HeLa cells), and 291/A2 (IgG1 anti-HCV e2 prepared from amino acids 384-715 of e2 in CHO cells) available from Bio-Chilie, and 472.2.5 (anti-HCV e2 prepared from e2 HV peptide) available from Mimotopes,

and 6A1 (IgG1 anti-HCV e2 prepared from amino acids 1-967 of e1/e2 in CHO cells; blocks binding to MOLT4 receptor) and 6A21 (IgG1; blocks binding to MOLT4 receptor) available from Biocine. Preferred monoclonal antibodies to linear epitopes include, but are not limited to, 3D5/C3 (IgG1 anti-HCV e1 prepared from amino acids 1-967 of e1/e2 in HeLa cells) and 3E5-1 and 3E5-2 (IgG1 anti-HCV e2 prepared from amino acids 404-661 of e2 in insect cells) available from Bio-Chilie. Additional monoclonal antibodies can be prepared as described herein using methods well known to the skilled artisan.

The presence of the immune complexes between the envelope proteins in the biological sample and the monoclonal antibodies is detected using any number of means widely known to the skilled artisan. For example, detectably labeled secondary antibodies can be added to the sample mixtures which react with the monoclonal antibodies. Preferably, the monoclonal anti-hepatitis envelope protein antibodies are detectably labeled. The labels may be, for example, enzymatic, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the immune complex are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

In preferred embodiments of the invention, biotin-streptavidin is used to detect the immune complexes. Preferably, the mouse monoclonal antibodies are biotinylated. Streptavidin conjugated to DMAE is added to the biological sample mixture in order to detect the biotinylated monoclonal antibodies. In other preferred embodiments of the invention, the sensitivity of the detection system can be enhanced by adding streptavidin conjugated to horseradish peroxidase (HRP) and subsequently adding secondary antibodies directed to HRP and conjugated to DMAE. A preferred secondary antibody is goat anti-HRP conjugated to DMAE. Other suitable detectable labels can be substituted for DMAE as known to those skilled in the art.

Another aspect of the present invention is directed to a method for screening blood components or blood for hepatitis C virus prior to the use of such blood or blood component to prepare blood products. In preferred embodiments of the invention, the method comprises reacting a body component from a potential donor with an anti-human antibody and at least one monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between the antibodies and the body component and detecting

the presence of immune complexes formed between the antibodies and hepatitis C virus envelope proteins. Preferably, any blood or blood component from the donor is discarded if the complexes are detected. The method of screening blood and blood products is essentially the same as for detecting the presence of HCV in biological samples.

In cases of a positive reactivity to the HCV antigen, it is preferable to repeat the immunoassay to lessen the possibility of false positives. For example, in the large scale screening of blood for the production of blood products (e.g., blood transfusion, plasma, Factor VIII, immunoglobulin, etc.), "screening" tests are typically formatted to increase sensitivity (to insure no contaminated blood passes) at the expense of specificity; i.e., the false-positive rate is increased. Thus, it is typical to only defer for further testing those donors who are "repeatedly reactive"; i.e., positive in two or more runs of the immunoassay on the donated sample.

The present invention is also directed to using the antibodies of the invention in connection with the HCV e1 and/or e2 detection system described herein to monitor treatment of individuals infected with HCV. An individual having HCV, for example, can be receiving conventional therapy, *i.e.*, interferon treatment. Such individuals, at some time in the treatment course, would be expected to undergo a relapse in HCV due to, for example, clearance of interferon or other drugs from the individual's body. One skilled in the art can monitor the amount of HCV e1 or e2 protein in the individual by the methods described above and be able to predict when such relapse may occur sooner than would be possible using currently available techniques for detection of HCV which focus on antibody detection. Thus, one skilled in the art would be able to begin a second round of drug therapy at an earlier date.

The present invention is also directed to kits for detecting hepatitis C virus in a biological sample. The kits preferably comprise anti-human antibody, at least one monoclonal anti-hepatitis C virus envelope protein antibody, and antibody control standards. Other kit components, such as, for example, instructions for use of the kit components can also be included. Preferred kits optionally comprise polyclonal anti-hepatitis C virus envelope protein antibody. Preferably, the anti-human antibody is attached to a solid phase. In preferred kits, the monoclonal antibody reacts with an epitope selected from the group consisting of an e2 conformational epitope, an e2 linear epitope, an e2 neutralizing epitope, e1 conformational epitope, an e1 linear epitope, and an e1 neutralizing epitope. Other

preferred kits comprise a plurality of monoclonal antibodies which react with an e2 conformational epitope, an e2 linear epitope, an e2 neutralizing epitope, e1 conformational epitope, an e1 linear epitope, an e1 neutralizing epitope, or a combination thereof. Preferably, the monoclonal antibody is detectably labeled as described above.

The following examples are intended to illustrate the invention and are not intended to limit the invention in any manner.

EXAMPLES

Example 1: Construction of PSC59 Poly

The HCV sequence used for the production of E1 and E2 was isolated from plasmid pC5P-1 as a StuI partial/BglII fragment. This fragment extends from the first methionine of HCV-1 polyprotein to aspartic acid at position 966. The domains included are the nucleocapsid, C, both putative envelope glycoproteins, E1 and E2, and a truncated form of NS2, respectively. In addition, the fragment also contains about 60 bp corresponding to that portion of the 5'-untranslated region of the HCV genome. One skilled in the art can also prepare other fragments containing portions of E1 and E2 as desired.

The fragment was treated with Klenow polymerase to create blunt ends, and then cloned into the StuI site of a vaccinia vector, PSC59 (obtained from Dr. B. Moss at the National Institutes of Health, Bethesda, Md); other vectors can be used, however. As a result of the ligation into the polylinker sequence of the vector, the C'-terminus of the NS2 region contains an additional Pro-Tyr sequence.

Example 2: Preparation of Stocks of Vaccinia Virus Encoding the HCV Polyprotein Fragment Including E1 and E2

The screening for recombinant Vaccinia virus was carried out essentially as described by Mackett et al. in DNA Cloning, Vol. II (Ed. D.M. Glover, IRL Press, Oxford, England, 1985, pp. 191-211). More specifically, a confluent monolayer (6 cm dish) of African green monkey kidney cells, BSC40, was infected with wild type Vaccinia virus (WR strain) at a multiplicity of infection (MOI) of 0.05. After a 2 hour incubation at 37° C, the cells were transfected with 25 μg of PSC59 poly DNA using the calcium phosphate method. After 4 hours of incubation, the medium was changed to normal medium, and the cells were

incubated for an additional 48 hours at 37° C. The cells were recovered by scraping them from the dish, and the virus was released by 3 cycles of freezing-thawing, and the released virus in the cell lysate were stored at -80° C.

In order to screen for recombinant virus, a confluent monolayer of human 143 TK$^-$ cells were infected for 2 hours with the cell lysate in 10-fold serial dilutions. After removal of the inoculum, 1% agarose in serum medium containing 25 µg/ml 5-bromodeoxyuridine was added, and the cells were incubated 72 hours at 37° C. Plaques were visualized by overlaying the cell layer with 1% agarose plus 0.01% neutral red, and incubating the cells overnight at 37° C. The agarose overlay was then carefully removed, and the cell layer was blotted with a master nitrocellulose filter (S&S, BA85, 0.45µm). A replica plate of the master filter was made, and probed with a ^{32}P-labeled hybridization probe to the HCV sequence. Positive plaques were isolated from the master filter, placed in 0.5 ml serum-free medium, and sonicated twice for 30 seconds. The screening process was repeated twice to plaque purify the virus.

In order to propagate the recombinant Vaccinia virus, ten dishes (150 cm^2) of BSC40 cells were infected with the viral stock at a MOI of 0.5. The infection was carried out for 2 hours at 37° C, and the viral stock replaced with fresh medium. After 72 hours the cells were harvested, suspended in 10 mM Tris HCl, Ph 9.0, and homogenized in a Wheaton dounce tissue grinder. Cell debris was removed by centrifugation, the supernatants were trypsinized and sonicated, and aliquots of the viral suspensions were stored at -80° C.

Example 3: Production of E1/E2 Antigens

One liter of Hela S3 spinner cells were brown in a spinner flask to a density of 10^6 cells per ml. The cells were infected with the recombinant Vaccinia virus encoding the HCV polyprotein fragment using a MOI of 1.0, incubated overnight, harvested, and stored as a cell pellet at -80° C.

The E1/E2 expression product was purified by lysing the cells in hypotonic buffer, followed by extraction in a buffer containing nonionic detergent. The cellular extract was chromatographed through a lectin (GNA) agarose column. The desired proteins were eluted from the column with methyl-α-D-mannopyranoside (Sigma Corp.). The eluted fractions were monitored for E1 and E2 by Western blots using a specific antiserum raised

against E1 or E2. The fractions containing the antigens were pooled and concentrated on a S-Sepharose column (Pharmacia). The purity of the final product was about 70%.

The E1 (130aa) and E2 (251aa) proteins can also be expressed as internal antigens within yeast, *S. cerevisiae*, as C-terminal fusions with human superoxide dismutase (SOD) using methods described previously by Kuo *et al.*, *Science*, **1989**, *244*, 362-364, and Cousens *et al.*, *Gene*, **1987**, *61*, 265-272, each of which is incorporated herein by reference in its entirety. Following cell breakage and centrifugation, the insoluble SOD fusion polypeptides were extracted from the cell pellets using either 5 M urea or 1% SDS and purified using either gel filtration or a combination of ion-exchange chromatography (Q- and S-sepharose) and gel filtration chromatography (Sephacryl S-300 HR).

The HCV native E1 and E2 antigen can also be purified from the endoplasmic reticulum of recombinant Vaccinia virus (rvv)-infected cells that contain the full length HCV E1 and E2 genes. Purification can be accomplished by affinity chromatography, followed by ion exchange chromatography under non-denaturing conditions as described in, for example, WO 92/08734 and in U.S. Serial No. 07/758,880, each of which are incorporated herein by reference in its entirety.

The native HCV E2 antigen, CHO-e2, can also be prepared essentially according to Spaete *et al.*, *Virology*, **1992**, *188*, 819-830, which is incorporated herein by reference in its entirety. More specifically, the mammalian CHO cell line producing CHO-e2 antigen is constructed from a plasmid containing an HCV-1 sequence encoding Ala383 to Glu661. The plasmid is then transfected into CHO cells to generate a stable line expressing full length e2 (also called e2/ns1) antigen. Clones exhibiting high expression are selected and expanded in roller bottles by growth in DME/H21 with 10% dialyzed fetal calf serum and the usual supplements plus 1.6 μM Methotrexate. The culture medium supernatant is harvested, and used for the purification of the CHO-e2 antigen. The purification scheme includes affinity and ion exchange chromatography under non-denaturing conditions.

In order to perturb the native e2 putative conformational epitopes, denatured CHO-e2 is prepared by addition of DL-dithiothreitol (DTT) to a final concentration of 10 mM, 0.2% sodium dodecyl sulfate (SDS), and boiled at 100°C for 5 minutes. All purified recombinant HCV antigens are at least 90% pure by SDS polyacrylamide gel analysis and staining with Coomassie blue.

Example 4: Detection Assay For Recombinant CHO-e2 Antigen

The sensitivity of a preferred detection assay was measured using recombinant CHO-e2 antigen. Briefly, a polyclonal chimp anti-HCV e1/e2 antibody/mouse anti-human IgG/PMP complex was contacted with recombinant HCV e2 produced in CHO cells as described above. A plurality of monoclonal anti-HCV envelope protein antibodies (291/A2, anti-e2 conformational; 1G2A7, anti-e2 neutralizing; and 3E5-2, anti-e2 linear) at 100 ng per assay were contacted to the complex. The monoclonal antibodies were labeled with biotin and the streptavidin system was used to measure binding. Typical results are shown in Table 1. The data indicates that the assay can detect CHO e2 antigen at concentrations as low as 1.95 ng/ml.

Table 1

ng/ml CHO e2	s (RLU)	s/n
500	1,048,879	374.2
250	528,574	188.6
125	298,991	106.7
62.5	147,963	52.8
31.3	80,558	28.8
15.6	43,166	15.4
7.81	23,254	8.3
3.91	14,060	5.0
1.95	8,870	3.2
0	2,803	1

s/n is the sensitivity (s) in relative light units (RLU) divided by the average negative value (s at 0 ng/ml of e2).

Example 5: Detection Assay For HCV With Clinical Biological Samples

A preferred detection assay which detects HCV e2 was compared to a detection assay which detects anti-HCV antibodies. Biological samples (serial bleeds) were obtained from two patients at several time points, for example, on 4/17 (I-1), 5/10 (I-2), 5/24 (I-3), 6/8 (I-4), 6/28 (I-5), 7/19 (I-6), and 12/28 (I-7). Patient I was an individual who had not yet

seroconverted. Patient Y had already seroconverted. Each sample was examined for the presence of HCV e2 protein (Protein Assay) or for the presence of anti-HCV antibodies (Antibody Assay). For the Antibody Assay, CHO e2 protein linked to a solid phase was used to capture human anti-HCV antibodies that were present in the biological samples. A biotinylated monoclonal anti-human IgG/streptavidin system was used to detect the presence of the human anti-HCV antibodies. For the Protein Assay, solid phase linked to an anti-human IgG linked to a polyclonal anti-e1e2 antibody, as described above, was contacted with the biological sample. This system is able to detect free HCV antigen as well as HCV antigen/human Ig immunocomplexes. A biotinylated anti-e2 antibody, such as, for example, a combination of 3E5-2 and 291/A2, was used along with streptavidin to detect the presence of the HCV e2 protein. Typical results are shown in Table 2 (Antibody Assay), Table 3 (Protein Assay) and Table 4 (Standard Controls).

Table 2: Antibody Assay

Patient/Sample	s (RLU)	s/n
I-1	3865	1.29
I-2	4666	1.56
I-3	6884	2.29
I-4	5159	1.72
I-5	4297	1.43
I-6	8131	2.71
I-7	19589	6.53
Y-1	10842	3.61
Y-2	32094	10.7
Y-3	84715	28.24
Y-4	93047	31.02
Y-5	97343	32.45
Y-6	75029	25.01
Y-7	77524	25.84

Table 3: Protein Assay

Patient/Sample	s (RLU)	s/n
I-1	3619	1.17
I-2	2926	0.95
I-3	3111	1.01
I-4	2372	0.77
I-5	2710	0.88
I-6	108647	35.1
I-7	7962	2.57
Y-1	3265	1.05
Y-2	3758	1.21
Y-3	3496	1.13
Y-4	3080	1.00
Y-5	3034	0.98
Y-6	3496	1.13
Y-7	4805	1.55

Table 4: Standard Controls

Standard CHO e2 (ng/ml)	s (RLU)	s/n
500	729082	235.6
250	354724	114.6
125	179610	58.0
62.5	102241	33.0
31.3	55640	18.0
15.6	30246	9.8
7.81	17125	5.5
3.91	10872	3.5

1.95	7623	2.5
0	3095	1.0
SAC	40471	13.1
NHS	5960	1.9

s/n is the sensitivity (s) in relative light units (RLU) divided by the average negative value (s at 0 ng/ml of e2).
SAC is a biological sample from a patient known to be infected with HCV and serves as a positive control.
NHS is normal human serum and serves as a negative control.

As can be seen from Tables 2 and 3, one skilled in the art using the HCV envelope protein detection system described above can detect HCV e2 protein earlier (bleed 6) than detection of human anti-HCV antibodies (bleed 7).

Figure 2 is a graph representing the treatment regimen for patient I described above. Blood sample were also taken in order to detect the presence of AST and ALT, liver enzymes which indicate the presence of liver damage. The biological samples (bleeds) were examined for the presence of HCV protein (dashed line) and human anti-HCV antibodies (solid line). Also indicated in Figure 2 is the timing of treatment with beta interferon and alpha interferon. As can be seen, as soon as liver damage was detected by monitoring the levels of AST and ALT, the patient received beta interferon (300 IU twice daily) for the period indicated. However, when the patient no longer received beta interferon treatment, HCV reinfection occurred as indicated by the presence of elevated levels of ALT and AST as well as by a dramatic increase in the detection of HCV envelope protein. In contrast, the increase in the level of human anti-HCV antibodies lagged dramatically. Upon detection of HCV envelope protein, the patient was promptly treated with alpha interferon which resulted in a subsidence in the HCV infection. Thus, the results depicted in Figure 2 demonstrate that detection of HCV envelope protein is a dramatic improvement over detection of human anti-HCV antibodies in biological samples and also shows the impact of the detection system on monitoring convention HCV treatment.

Example 6: Detection Assay For Immunocomplexed HCV

The detection system described herein can also be used to detect immunocomplexed HCV envelope antigens. A preferred detection assay was performed to detect varying amounts of immunocomplexed HCV envelope antigen in several biological samples. Biological samples included I-6 (from Example 5; no immunocomplex), SAC (from Example 5; light immunocomplex), Y-7 (from Example 5; medium immunocomplex), and JP (sample from a patient with high seroconversion; heavy immunocomplex). Each sample was examined for the presence of HCV e2 protein (Protein Assay) as described above in Example 5 or for the presence of anti-HCV antibodies (Antibody Assay) as described above for Example 5. Typical results are shown in Table 5.

Table 5

	Antibody Assay		Protein Assay	
Biological Sample	s (RLU)	s/n	s (RLU)	s/n
I-6	8131	2.78	108647	51.8
SAC	19000	6.49	40471	19.3
Y-7	73524	25.13	8963	4.3
JP	108770	37.17	1817	0.9

s/n is the sensitivity (s) in relative light units (RLU) divided by the average negative value (s at 0 ng/ml of e2).

Example 7: Enhanced Detection Assay For HCV Envelope Proteins

The assay sensitivity of the detection system described herein can be amplified by applying signal amplification to a current assay format. For example, mouse monoclonal antibody to HCV e2 protein (5E5/H& adsorbed) linked to superparamagnetic latex particles (Estapor) was used to capture varying amounts of CHO e2 protein. Biotinylated polyclonal antibody to HCV e1e2 was used to detect the CHO e2 protein. A streptavidin-horse radish peroxidase (hrp) complex was used to bind to the biotinylated polyclonal antibody to HCV e1e2. In addition, a DMAE conjugated anti-hrp antibody (from various animals) was used to detect the streptavidin-horse radish peroxidase (hrp) complex, thus amplifying the signal. Typical results are shown in Table 6.

Table 6

	goat anti-hrp		rabbit anti-hrp		mouse anti-hrp		rat anti-hrp	
CHO e2 (ng/ml)	s (RLU)	s/n	s (RLU)	s/n	s (RLU)	s/n	s (RLU)	s/n
500	766335	178.34	259952	116.41	3650	1.90	164487	52.87
250	415785	96.76	165889	74.29	4204	2.18	117810	37.87
125	317810	73.96	122430	54.83	3496	1.82	54162	17.41
62.5	127774	29.74	57242	25.63	1956	1.02	39747	12.78
31.3	70825	16.48	37961	17.00	2510	1.30	24224	7.79
15.6	48833	11.36	15169	6.79	2079	1.08	10657	3.43
7.81	24532	5.71	9317	4.17	3819	1.98	9440	3.03
3.91	8177	1.90	6699	3.00	3557	1.85	5606	1.80
1.95	5652	1.32	4481	2.01	3450	1.79	5159	1.66
0	4297	1.00	2233	1.00	1925	1.00	3111	1.00

s/n is the sensitivity (s) in relative light units (RLU) divided by the average negative value (s at 0 ng/ml of e2).

The sensitivity can be increased even further by using paramagnetic particles (PMP) instead of magnetic latex particles (MLP) and higher affinity anti-hrp second antibody conjugate (DMAE).

What is Claimed is:

1. A method for detecting hepatitis C virus in a biological sample comprising the steps of:

contacting said sample with an anti-human antibody and at least one monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between said antibodies and said sample; and

detecting the presence of immune complexes of said antibodies and said envelope protein.

2. The method of claim 1 wherein said anti-human antibody is attached to a solid phase.

3. The method of claim 2 wherein said solid phase is selected from the group consisting of microtiter plates, paramagnetic particles, and paramagnetic beads.

4. The method of claim 1 wherein said monoclonal antibody reacts with an epitope selected from the group consisting of an e2 conformational epitope, an e2 linear epitope, an e2 linear neutralizing epitope, e1 conformational epitope, an e1 linear epitope, and an e1 linear neutralizing epitope.

5. The method of claim 1 wherein said at least one monoclonal antibody reacts with an e2 conformational epitope, an e2 linear epitope, an e2 linear neutralizing epitope, e1 conformational epitope, an e1 linear epitope, an e1 linear neutralizing epitope, or a combination thereof.

6. The method of claim 1 wherein said monoclonal antibody is detectably labeled.

7. The method of claim 1 wherein said anti-human antibody is contacted with a polyclonal anti-hepatitis C virus envelope protein antibody prior to contact with a biological sample.

8. A method for detecting hepatitis C virus in a biological sample comprising:

contacting an anti-human antibody attached to a solid phase with a polyclonal anti-hepatitis C virus envelope protein antibody;

contacting said sample to said polyclonal antibody;

contacting said sample with at least one detectably-labeled, monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between said antibodies and said sample; and

detecting the presence of immune complexes of said antibodies and said envelope protein.

9. A method of screening blood components or blood for hepatitis C virus prior to the use of such blood or blood component to prepare blood products comprising:

reacting a body component from a potential donor with an anti-human antibody and at least one monoclonal anti-hepatitis C virus envelope protein antibody under conditions that allow an immunologic reaction between said antibodies and said body component;

detecting the presence of immune complexes formed between said antibodies and hepatitis C virus envelope proteins; and

discarding any blood or blood component from said donor if said complexes are detected.

10. A kit for detecting hepatitis C virus in a biological sample comprising:

an anti-human antibody;

at least one monoclonal anti-hepatitis C virus envelope protein antibody;

control standards; and

instructions for use of the kit components.

11. The kit of claim 10 further comprising a polyclonal anti-hepatitis C virus envelope protein antibody.

12. The kit of claim 10 wherein said anti-human antibody is attached to a solid phase.

13. The kit of claim 10 wherein said monoclonal antibody reacts with an epitope selected from the group consisting of an e2 conformational epitope, an e2 linear epitope, an e2 linear neutralizing epitope, e1 conformational epitope, an e1 linear epitope, and an e1 linear neutralizing epitope.

14. The kit of claim 10 comprising a plurality of monoclonal antibodies which react with an e2 conformational epitope, an e2 linear epitope, an e2 linear neutralizing epitope, e1 conformational epitope, an e1 linear epitope, an e1 linear neutralizing epitope, or a combination thereof.

15. The kit of claim 10 wherein said monoclonal antibody is detectably labeled.

Assay Configurations

FIG. 1A

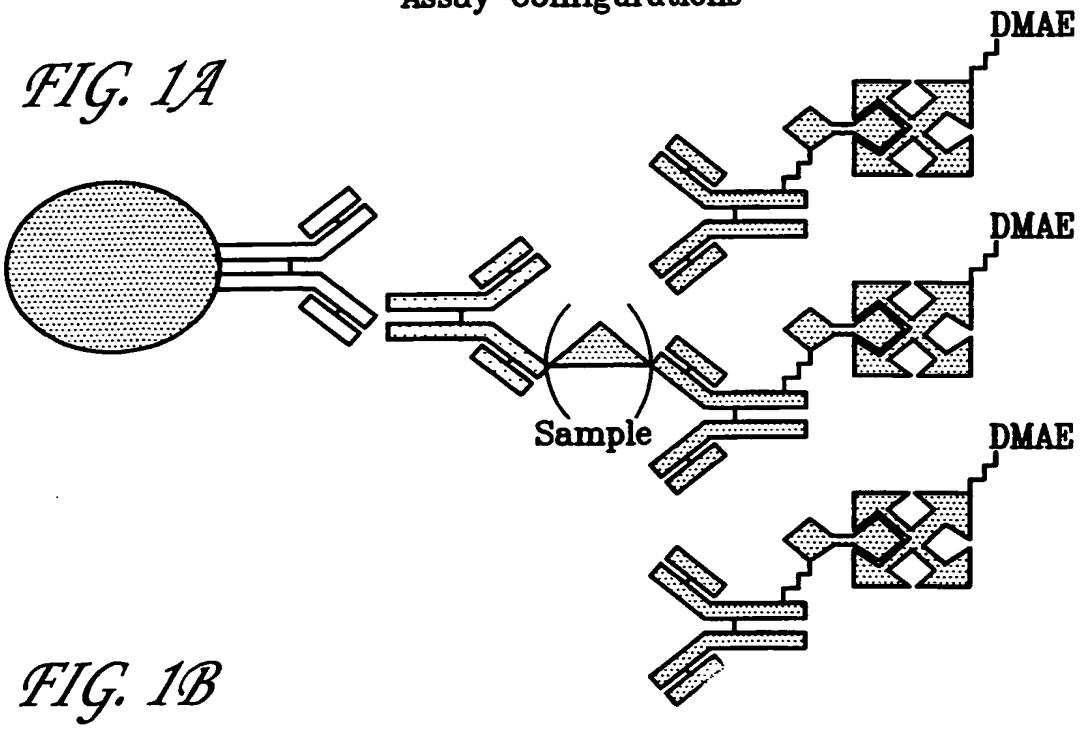


FIG. 1B

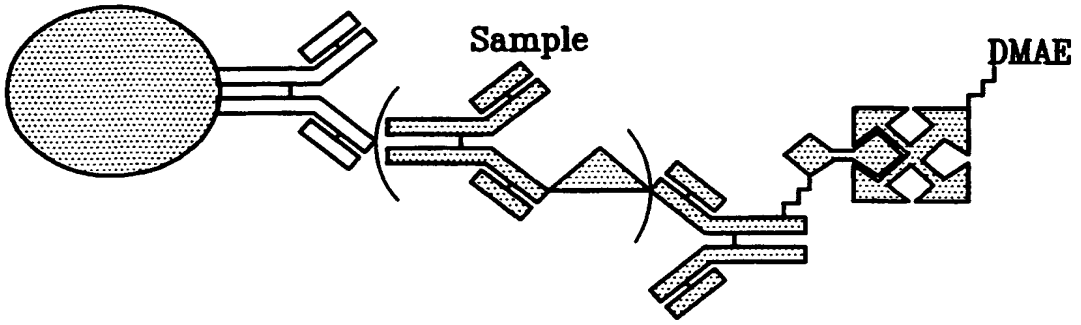


FIG. 1C

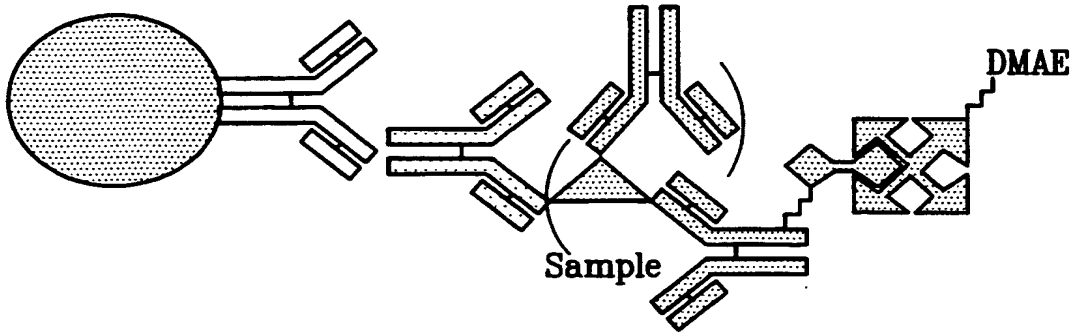
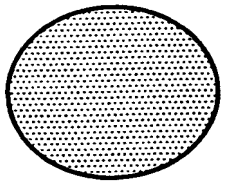
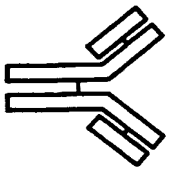


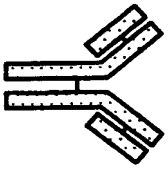
FIG. 1D



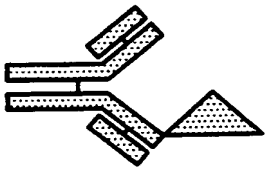
= Paramagnetic Particle



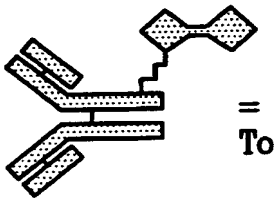
= Mouse Anti-Human IgG



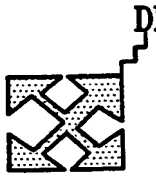
= Polyclonal Antibody to HCV e1/e2



= HCV Positive Sample
Human IgG Anti-HCV Immunocomplex



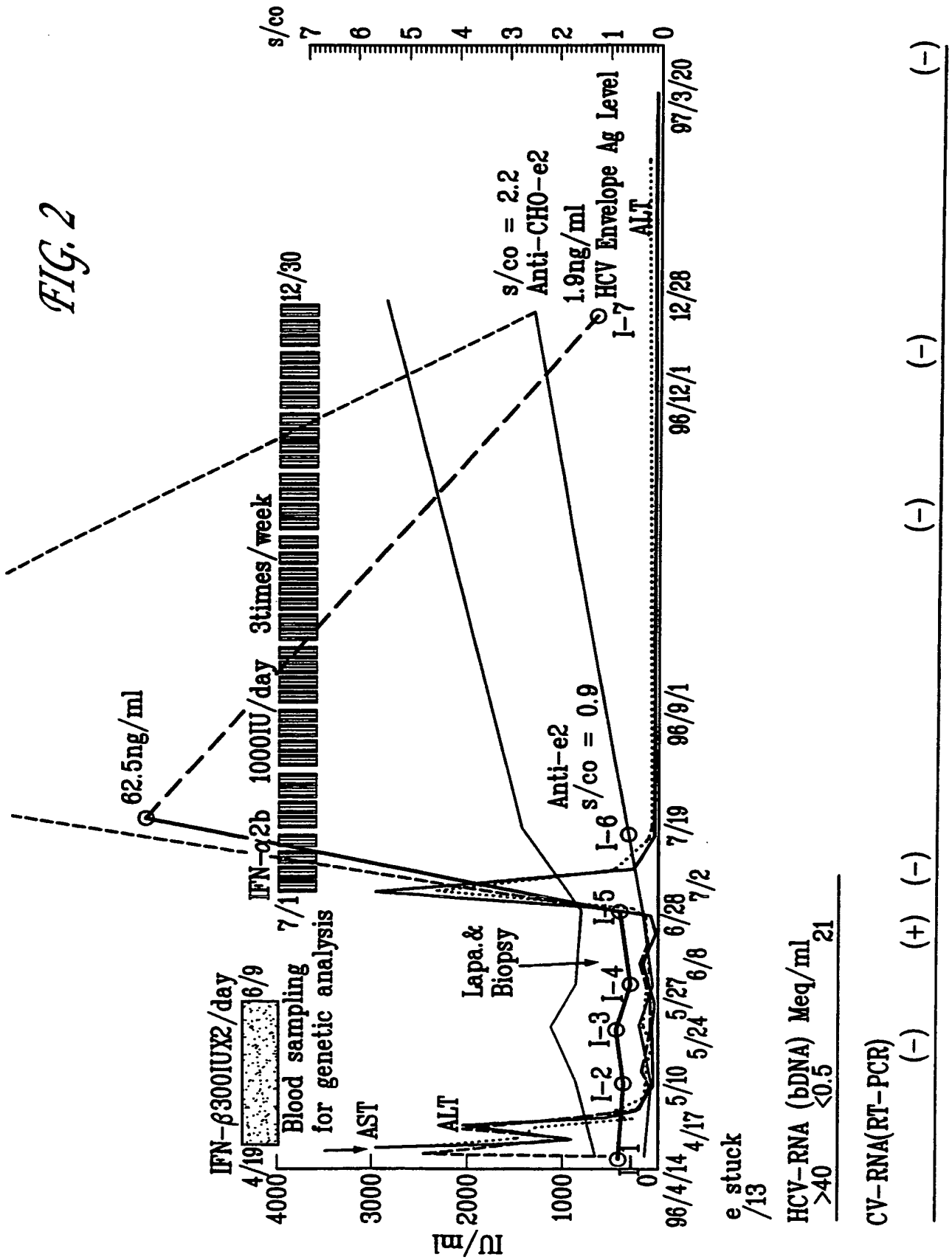
= Mouse Monoclonal Antibody
To HCV e2 Biotinylated



= Streptavidin Conjugated to DMAE

= Ag

FIG. 2


s/co
7
6
5
4
3
2
1
0
IU/ml
4000
3000
2000
1000
0
62.5ng/ml
IFN-α2b 1000IU/day 3times/week
7/1
12/30
IFN-β300IUX2/day
4/19
6/9
Blood sampling for genetic analysis
AST
ALT
Lapa.& Biopsy
I-1
I-2
I-3
I-4
I-5
I-6
I-7
s/co = 2.2
Anti-CHO-e2
1.9ng/ml
HCV Envelope Ag Level
ALT
Anti-e2
s/co = 0.9
96/4/14
4/17
5/10
5/24
5/27
6/8
6/28
7/2
7/19
96/9/1
96/12/1
12/28
97/3/20
e stuck
/13
HCV-RNA (bDNA) Meq/ml
>40
<0.5
21
CV-RNA(RT-PCR)
(-) (+) (-) (-) (-) (-)

10/048,191

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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Filed on 28 July 1999 (28.07.1999)

(71) Applicant *(for all designated States except US)*: **CHIRON CORPORATION** [US/US]; 4560 Horton Street, Emeryville, CA 94608 (US).

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(75) Inventors/Applicants *(for US only)*: **CHIEN, David, Y.** [US/US]; 1121 Douglas Court, Alamo, CA 94507 (US). **ARCANGEL, Phillip** [US/US]; Apt. 303, 2541 Regent Street, Berkeley, CA 94704 (US).

(74) Agent: **HARBIN, Alisa, A.**; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).

(81) Designated States *(national)*: CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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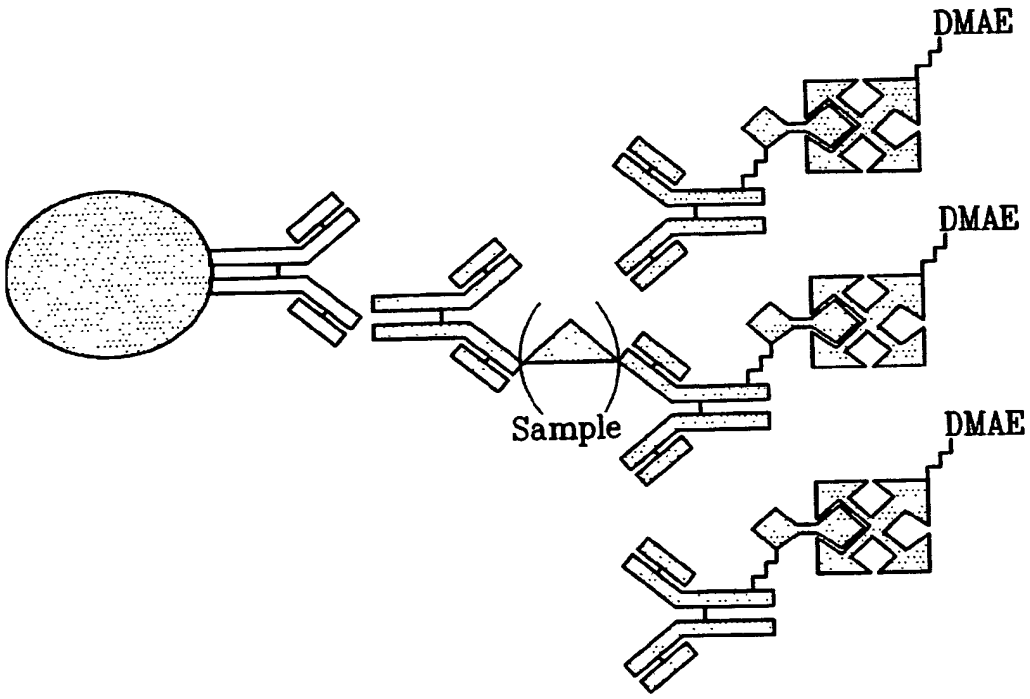
(54) Title: HEPATITIS C VIRAL ANTIGEN IMMUNOASSAY DETECTION SYSTEMS



(57) Abstract: Immunoassays for detecting hepatitis C virus protein and immune complexes between hepatitis C virus protein and antibodies in biological samples, methods of screening blood products for hepatitis C virus, and kits employed therefor are provided.

WO 01/009609 A3

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Ir onal Application No
PCT/US 00/20214

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/576 G01N33/543 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 40176 A (ALLANDER TOBIAS ERIK ;PERSSON MATS AXEL ATTERDAG (SE)) 30 October 1997 (1997-10-30) page 5, line 21-30; claims 1,10,33,36 page 53, line 15 -page 54, line 30 page 56, line 4-26 ---	1-15
Y	WO 93 04205 A (ABBOTT LAB) 4 March 1993 (1993-03-04) page 3, line 11 -page 4, line 2 page 7, line 20-30; claims 11,13,14,17,18,21; example 7 ---	1-15
Y	WO 92 13892 A (ABBOTT LAB) 20 August 1992 (1992-08-20) page 7, line 25-30; claims 11,15,16,20,21,24,34 page 12, line 28-33 ---	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 October 2002	29/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vadot-Van Geldre, E

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International Application No
PCT/US 00/20214

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 93 06488 A (GENELABS TECH INC) 1 April 1993 (1993-04-01) page 10, line 31 -page 11, line 30 page 12, line 13-25 page 41, line 20-35 page 43, line 7-9 page 45, line 33 -page 46, line 2 page 55, line 13-30; examples 19,20; table 3	1-15
Y	FR 2 502 154 A (TREPO CHRISTIAN) 24 September 1982 (1982-09-24) page 5 -page 9 page 11 -page 13 page 15 page 29 -page 30	1-15
Y	US 4 464 474 A (COURSAGET PIERRE L J ET AL) 7 August 1984 (1984-08-07) claims 7,8; example 8	1-15
Y	WO 94 01778 A (CHIRON CORP) 20 January 1994 (1994-01-20) page 2, line 25-28 page 6, line 2-6 page 13, line 12-19; claims; example 4	1-15
Y	WO 99 15898 A (CHIRON CORP) 1 April 1999 (1999-04-01) page 2, line 15-20 page 4, line 23-25 page 5, line 10-23; figure 1; example 1	1-15

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/US 00/20214

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9740176	A	30-10-1997	US	2002016445 A1	07-02-2002
			CA	2262423 A1	30-10-1997
			WO	9740176 A1	30-10-1997
			EP	0937153 A1	25-08-1999
			JP	2000509966 T	08-08-2000
WO 9304205	A	04-03-1993	US	5308750 A	03-05-1994
			AT	185606 T	15-10-1999
			AU	2585092 A	16-03-1993
			CA	2115923 A1	04-03-1993
			DE	69230146 D1	18-11-1999
			DE	69230146 T2	25-05-2000
			EP	0603307 A1	29-06-1994
			ES	2139607 T3	16-02-2000
			JP	6510192 T	17-11-1994
			WO	9304205 A1	04-03-1993
WO 9213892	A	20-08-1992	AT	167522 T	15-07-1998
			AU	662517 B2	07-09-1995
			AU	1362792 A	07-09-1992
			CA	2101534 A1	01-08-1992
			DE	69225960 D1	23-07-1998
			DE	69225960 T2	21-01-1999
			DK	569537 T3	06-04-1999
			EP	0569537 A1	18-11-1993
			ES	2118129 T3	16-09-1998
			HK	1012838 A1	06-08-1999
			JP	6505389 T	23-06-1994
			WO	9213892 A1	20-08-1992
WO 9306488	A	01-04-1993	AU	2683792 A	27-04-1993
			WO	9306488 A1	01-04-1993
FR 2502154	A	24-09-1982	FR	2502154 A1	24-09-1982
			AT	15605 T	15-10-1985
			DE	3266294 D1	24-10-1985
			EP	0074986 A1	30-03-1983
			WO	8203330 A1	14-10-1982
US 4464474	A	07-08-1984	DE	3175439 D1	13-11-1986
			EP	0058676 A1	01-09-1982
			IT	1138449 B	17-09-1986
			WO	8200205 A1	21-01-1982
WO 9401778	A	20-01-1994	AT	216779 T	15-05-2002
			AU	685059 B2	15-01-1998
			AU	4662993 A	31-01-1994
			CA	2139645 A1	20-01-1994
			CZ	9500006 A3	13-03-1996
			DE	69331845 D1	06-06-2002
			DE	69331845 T2	14-08-2002
			DK	649537 T3	17-06-2002
			EP	0649537 A1	26-04-1995
			FI	950002 A	27-02-1995
			HU	70473 A2	30-10-1995
			JP	7509060 T	05-10-1995
			NO	950006 A	24-02-1995
			PL	307178 A1	02-05-1995

INTERNATIONAL SEARCH REPORT

Int ional Application No
P.., JS 00/20214

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9401778 A		RU 2126158 C1	10-02-1999
		SK 495 A3	11-07-1995
		WO 9401778 A1	20-01-1994
WO 9915898 A	01-04-1999	AU 9497998 A	12-04-1999
		AU 9498098 A	12-04-1999
		CA 2303123 A1	01-04-1999
		CA 2303165 A1	01-04-1999
		EP 1021719 A1	26-07-2000
		EP 1021723 A1	26-07-2000
		JP 2001517797 T	09-10-2001
		JP 2001517799 T	09-10-2001
		WO 9915898 A1	01-04-1999
		WO 9915901 A1	01-04-1999
		US 6391540 B1	21-05-2002
		US 6261764 B1	17-07-2001
		US 2001039009 A1	08-11-2001

RC545.H45

5/7/14
DIALOG(R)File 155:MEDLINE(R)

10166415 99162386 PMID: 10051497

Persistence of viremia and the importance of long-term follow-up after acute hepatitis C infection.

Villano S A; Vlahov D; Nelson K E; Cohn S; Thomas D L
Division of Infectious Diseases, Johns Hopkins School of Medicine, Baltimore, MD, USA.

Hepatology (Baltimore, Md.) (UNITED STATES) Mar 1999, 29 (3) p908-14 , ISSN 0270-9139 Journal Code: 8302946
Contract/Grant No.: AI-40035; AI; NIAID; DA-04334; DA; NIDA; DA-08004; DA ; NIDA; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The purpose of this investigation was to prospectively characterize acute hepatitis C virus (HCV) infections and to evaluate the hypothesis that the outcome is affected by identifiable clinical or viral factors. One hundred forty-two people with a history of illicit drug use who were HCV antibody-negative in 1988 were followed semiannually through 1996. HCV seroconversion (second generation enzyme immunoassay and recombinant immunoblot assay) was recognized in 43 (30%) of the participants, who were followed up for a median of 72 months. HCV RNA was detected and quantified by polymerase chain reaction in a median of 10 specimens per participant and showed two distinct patterns of viremia: viral clearance was noted in 6 (14%) of the participants, and viral persistence was observed in 37 (86%) of the participants. Subjects with viral clearance were more likely to be white (P =.004), have jaundice (P =.03), and have lower peak viral titer (P =.003). However, the outcome for a given person could not be predicted by clinical features, RNA level, or HCV subtype (as ascertained by analysis of core-E1 complementary DNA sequence). No acute infections were recognized by health care providers. At the time of seroconversion, HCV RNA was detectable in 81% of participants, and recombinant immunoblot assay (RIBA) was positive in 85% of participants. We conclude that approximately 85% of people with acute hepatitis C develop persistent viremia. However, acute infections are uncommonly recognized clinically, underscoring the importance of screening individuals at risk. Long-term follow-up, but no single laboratory test, is necessary to ascertain the outcome and in some cases make the diagnosis of acute HCV infection.

Record Date Created: 19990329

RD 120.7.765

5/7/15
DIALOG(R)File 155:MEDLINE(R)

10134227 99118773 PMID: 9921800

Antibodies to hepatitis C virus envelope proteins correlate with hepatitis C viraemia after liver transplantation.

Gane E J; Maertens G; Ducatteeuw A; Qian K P; Lau J Y; Jones H; Davies E; Naoumov N V; Williams R

Institute of Liver Studies, King's College School of Medicine and Dentistry, Denmark Hill, London, United Kingdom.

Transplantation (UNITED STATES) Jan 15 1999, 67 (1) p78-84, ISSN 0041-1337 Journal Code: 0132144

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

BACKGROUND: Liver transplant recipients for hepatitis C virus (HCV)-related cirrhosis usually remain anti-HCV-seropositive after transplantation. The aim of this study was to characterize, longitudinally, the profile of HCV-specific antibodies and cryoglobulins in liver transplant recipients with recurrent HCV infection. METHODS: Serial serum samples were collected prospectively before, at 1 month after, and at 12 months after transplantation for HCV cirrhosis in 30 patients infected with genotype 1. The antibodies against HCV envelope proteins (E1 and E2) were quantitated by enzyme-linked immunosorbent assay and antibodies against core, E2/hypervariable region I (HVRI), NS3, NS4, and NS5A antigens by a line immunoassay. Sera were also tested for cryoglobulins. RESULTS: The titer of each anti-HCV antibody had fallen at 1 month after transplantation ($P<0.05$) with the exception of anti-E1 levels, which had risen in 16 patients with acute hepatitis C at that time ($P=0.01$). Anti-E1 and anti-E2 titers, but not antibodies against other HCV antigens, increased to pre-transplantation levels or higher at 12 months, which correlated with serum HCV RNA levels. Cryoglobulinemia was present in nine patients after transplantation (30%) and was associated with lower anti-E1 levels ($P=0.04$) and more severe graft damage. CONCLUSIONS: The early increase in antibodies to HCV envelope proteins in correlation with viremia suggests that the envelope-specific humoral immune response may be directly stimulated by HCV replication. Anti-E1 levels may be a useful marker in monitoring patients with recurrent HCV infection.

Record Date Created: 19990222